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A mixture containing a nonvolatile acid, e.g., citric acid, and the salt of a volatile acid, e.g., sodium bicarbonate, was developed for the controlled generation of in-package carbon dioxide atmospheres during the refrigerated storage of wrapped fresh meat products. The dry chemical reactants, contained in packets and thus separated from contact with the meat product, form and release carbon dioxide as the moisture content within the package increases. This gas effectively retards the

growth of the principal meat spoilage organisms. The in-package gas release and concentration are functions of the amount and type of reactants, the free volume within the package, and the permeability of the different types of plastic overwrap. The method is effective with fresh, carefully handled meat and provides an additional safeguard against spoilage over normal processing. With other food products, similar or different reactant mixtures may be employed.

In the course of preliminary experiments on the effects of different chemical additives on meat color, it was noted that dilute bicarbonate solutions which had been applied to the meat surface either separately or along with ascorbic acid or citric acid solutions were effective in keeping the desired color of retail cuts for several days beyond untreated meat samples. A portion of this improvement was found to arise from the production of a covering carbon dioxide gas which reduced growth on the meat surface of *Pseudomonas* species, the common aerobic meat spoilage organism (King and Nagel, 1967). Various investigators (Pohja, 1968; Baran et al., 1970; Clark and Lentz, 1969, 1972) have noticed that carbon dioxide atmospheres similar to those used for storage of fruits and vegetables reduced surface slime formation and increased the keeping quality of the meat. Optimum results with meat color were obtained when carbon dioxide at concentrations of 10–15% was used in conjunction with high levels of oxygen (85%) (Clark and Lentz, 1973; Taylor and MacDougall, 1973; Naumann and Balasundaram, 1974). These findings have not been utilized maximally with fresh meat products because of the nature of processing and storage, which normally limits the total storage time to less than 3 weeks following slaughter, and the toxicity to humans of carbon dioxide levels of 10–15%, which would be present in the meat storage areas. Carbon dioxide as solid snow or pellets has been employed in the long distance shipping of meat within airtight containers to decrease spoilage during transport (Smith et al., 1974).

We have developed a system utilizing dry chemicals in packets to provide a continuous carbon dioxide generating system which can protect the carcass and cuts during storage when wrapped. The method uses GRAS substances, is inexpensive and practical, and can be adapted for market cuts of meat or for other perishable foods. The method involves the use of a mixture of two chemical reactants, a nonvolatile acid and the salt of a volatile acid, in dry form which are separated in packets from contact with the food and which react to form and release gaseous atmospheres as the moisture diffuses into the reagent packet.

EXPERIMENTAL SECTION

Materials and Methods. Sodium bicarbonate, citric acid, ascorbic acid, sodium bisulfate, sodium bisulfite, and sodium nitrite (all reagent grade) were purchased from commercial sources. Meat samples of known post-slaughter

history were taken from the longissimus dorsi or semitendinosus muscle after the carcass had been conditioned for 24 hr post-slaughter. In addition, eye round roasts (semitendinosus muscle) were purchased at commercial retail supermarkets and were trimmed of the external fat and surface before use. All meats were handled to minimize bacterial contamination. For experiments which involved the measurement of color by reflectance, the meat samples were trimmed to a size, 1 in. × 2 in. × 1.5 in., to fit the sample port of the spectrophotometer and were wrapped with FMC-MC commercial meat wrap film. MC meat wrap film is a stretch polyvinyl chloride film manufactured by FMC, Marcus Hook, Pa. For storage stability tests, meat samples wrapped in this film were stored in low-permeability polyethylene bags with the packets of chemicals enclosed.

For normal storage studies, the samples were maintained in a commercial meat display cooler at -1.1°C . The monitoring of the meat surface temperatures indicated a rise of approximately 2°C during defrost cycles or daylight illumination. A walk-in cooler maintained at 7.2°C was used for the storage of samples used in the examination of the effect of higher storage temperatures.

Calculated weights of the powders employed in the storage experiments were mixed together, and weighed aliquots were placed in porous cellulose fiber envelopes. The envelopes were prepared by hand from nonwoven disposable cellulose cloths, initially 18 in. × 18 in. Depending upon the porosity of the cloth and the thickness desired, up to 36 envelopes 1.5 in. × 3 in. could be prepared from a single sheet. The envelopes were held together and sealed at the edges with pressure sensitive tape, and could contain approximately 5 g of powder. Prepared envelopes containing powder were kept in a desiccator until use to prevent premature chemical reactions.

The viable bacterial count of the meat samples was determined by a standardized shaking method developed for these studies. The meat samples (ca. 50 g) were weighed under aseptic conditions and suspended in 4 weight vol of sterile 0.1% peptone (Difco) water in a 1-qt sterile Mason jar. The Mason jar was shaken intermittently for 2–3 min to remove the bacteria from the meat surfaces. After shaking, appropriate dilutions, also prepared with 0.1% peptone water, were surface plated in triplicate onto Nutrient Agar (Difco). The plates were counted after 72-hr incubation at 20°C . Total bacterial count was expressed as the logarithm of the mean count per gram. Individual colonies were isolated in some instances and characterized by gram staining and tested for the presence of catalase.

The reflectance spectra of meat samples were determined with a Beckman DB-G spectrophotometer equipped with an integrating sphere reflectance attachment. The

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Table I. Effective Acids for Gas Generation in Food Products

Acid	pK_a^a	Mol wt ^a	Effective value(s)
Nonvolatile			
Ascorbic	4.17	176	Inexpensive, free-radical trap
Benzoic	4.2	122	Lipophilic, delays reaction
Bisulfate ^b	1.92	120	Strong acid salt
Citric	3.1, 4.9, 5.7	192	Inexpensive, chelate, tribasic
Lactic	3.86	90	Present in meat fluid, nonsolid
Phosphoric	1.8, 6.85, 12	98	Present in meat fluid, tribasic, nonsolid
Pyruvic	2.6	88	May be present in meat fluid
Sorbic	4.8	122	Lipophilic, delays reaction
Volatile			
Acetic	4.76	82 ^c	Used as meat germicidal rinse
Carbonic	6.1, 9.9	84 ^c	Reduces Pseudomonad growth, dibasic
Formic	3.75	68 ^c	Unknown, may be similar to acetic
Hypochlorous	7.46	74 ^c	Germicidal
Nitrous	3.29	69 ^c	Prevents Clostridial toxin formation
Propionic	4.87	96 ^c	Mycostat
Sulfurous	1.76, 7.2	104 ^c	Mycostat, germicide

^a Reference, Handbook of Chemistry and Physics, 1967. ^b All acids listed with the exception of bisulfate are GRAS; bisulfate is not permitted in foods as an additive, but may be present as a form of sulfate in high acid foods. ^c As sodium salt.

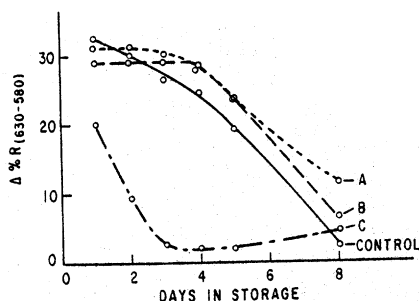


Figure 1. Effect of selected atmosphere treatments on the color stability of beef eye round muscle. Ordinate values indicate the degree of redness and are described more fully in the text. Higher values indicate increased redness. Meat samples were wrapped in PVC stretch film and were stored in plastic bags not in physical contact with packets containing chemical mixtures: (A) sodium bisulfate and sodium bisulfite (sulfur dioxide gas); (B) citric acid and sodium bicarbonate (carbon dioxide gas); (C) ascorbic acid and sodium nitrite (nitrogen oxides); control, no treatment.

machine was standardized with a magnesium carbonate block wrapped with the plastic meat film. The color of the meat samples was monitored daily by the method of Strange et al. (1974).

Carbon dioxide release from packets was examined manometrically using a Gilson differential respirometer equipped with large size manometric flasks (Aminco no. 5B210). These flasks could hold packets and meat samples separated for both short-term manometric analysis and long-term gas volume collection experiments. For the carbon dioxide release measurements, weighed amounts of a 4:5.2 mixture of citric acid-sodium bicarbonate were placed in the flask with 1.0 ml of water in the side arm. The side arm was not tipped but the presence of water vapor simulated the buildup of moisture within a package. The rate of carbon dioxide evolved during a 15-min period at 0°C was measured hourly.

RESULTS AND DISCUSSION

The reaction leading to the formation of a volatile product is of a general type, produced readily with mixtures of nonvolatile acids and salts of volatile acids. The rate and extent of this reaction may be controlled by selection of acids with appropriate pK_a values and hydrophilicity and

by variations in the amounts of acid and salt added. The list of materials which may be used for this purpose of gas generation in food products is limited by the requirements of safety and nontoxicity. Table I lists volatile and nonvolatile acids frequently used as food additives with their pK_a values, molecular weights, and effective value. For the reaction to go to completion, it is necessary to have a solid acid of sufficient acid strength and/or molar concentration to release the volatile acid. For the most effective action, with equal molar concentrations of acid and salt, the pK_a of the nonvolatile acid should be at least 2.0 pH units below the pK_a of the volatile acid, e.g., below pH 4.1 if bicarbonate is to be used as the acid salt. Differences in the concentration of the salt and the acid may be calculated by the Henderson-Hasselbach equation.

Although this system has been adapted specifically to maintain meat quality for longer periods of time through the generation of carbon dioxide atmospheres, the same type of system may be applied with minor variations to other food products. Hypochlorous acid as a sanitizer and sulfurous acid as a bleaching agent or fungicide are permitted with limitations for the preparation of certain foods, e.g., dried fruits. This system might find application with these other foods if permitted. Hypochlorous acid can be generated from hypochlorite salts and sulfurous acid can be generated from bisulfite or sulfite salts. However, with meat, the use of sulfurous acid is not permitted because of cosmetic effects on spoiled products, and hypochlorite solutions are permitted only experimentally for meat carcass sanitation. The carbonic acid salts, carbonate and bicarbonate, appear to offer the greatest advantage for meat in terms of cost and availability. In addition, a slight reaction may also be produced between solid sodium bicarbonate and lactic, pyruvic, or phosphoric acids which may be present as salts (Table I) in the meat drip fluid (pH 5.2-5.7).

The effect of selected treatments on the color stability of beef eye round muscle is shown in Figure 1. These samples were prepared from meat purchased from a supermarket and demonstrate the effectiveness of such treatments under nonoptimum conditions. The ordinate values reflect the degree of redness, with decreasing values indicating an alteration of the oxymyoglobin to metmyoglobin. Although there is no accepted procedure for measuring the rate of color change in meat products, values obtained by reflectance spectrophotometry of the meat products can provide

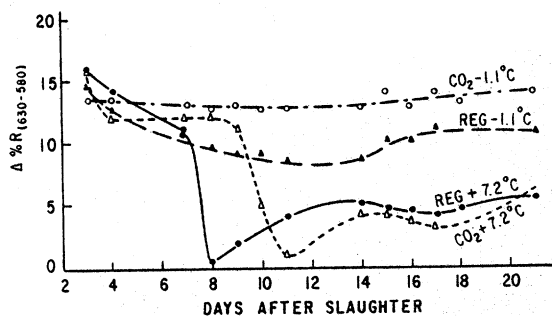


Figure 2. Effect of atmosphere and temperature on color stability of longissimus dorsi muscle in storage. Ordinate values as in Figure 1: (●—●) regular atmosphere, storage temperature 7.2°C; (▲—▲) regular atmosphere, storage temperature -1.1°C; (△---△) carbon dioxide atmosphere, storage temperature 7.2°C; (○---○) carbon dioxide atmosphere, storage temperature -1.1°C.

an indication of the myoglobin changes resulting from bacterial and/or oxidative changes. With the alteration of the heme pigment color from bright red to brown, the percent reflectance (% *R*) at 630 nm contributed by the red oxymyoglobin form decreases, and the % *R* at 580 nm contributed by the brown metmyoglobin form increases (Strange et al., 1974). With the alteration of the oxymyoglobin to metmyoglobin, the difference ($\Delta\% R = \% R \text{ at } 630 \text{ nm} - \% R \text{ at } 580 \text{ nm}$) decreases and approaches zero, and even negative values. This difference value shows a high linear correlation with consumer preference in meat color (Strange et al., 1974). Normally, the metmyoglobin form is produced as a result of surface bacterial growth, but such changes may also occur from a coupled reaction with meat lipid oxidations (Benedict et al., 1975). Some formation of metmyoglobin is also noted when meat is stored under high concentrations of carbon dioxide and very low concentrations of oxygen (Ledward, 1970), but this altered surface color may be reversed in part by subsequent flushing with oxygen.

The rapid change in color value of meat samples produced by the packets which contained the ascorbic acid-sodium nitrite mixture is from the oxidation of the surface myoglobin by the nitrogen oxides and/or other oxidants released in the chemical reaction and from the formation of nitrogen oxide myoglobin compounds. Although no contact between the meat and nitrite occurred, the reaction is similar to that occurring when nitrite is added directly to meat. The effectiveness of the bisulfite-sodium bisulfate mixture on maintaining the red color results from the production of sulfur dioxide gas which prevents the formation of metmyoglobin. Both the nitrite (nitrogen oxide gas) and bisulfite (sulfur dioxide gas) packets were extremely effective in reducing bacterial growth on the meat surface; bacterial growth was not detected (less than 100/g) during 10-days storage with either treatment, indicating that these treatments might have value for special applications. The bicarbonate-citric acid packet (carbon dioxide gas) showed some value in extending the color over the untreated controls by the regulation of the surface bacterial growth. After 11-days storage the bacterial counts of the bicarbonate-citric acid mixture (carbon dioxide) treated samples were similar to those of the untreated controls, indicating that this method could not be used to conceal meat of high bacterial contamination. The atmosphere with increased carbon dioxide levels acts only to slow down surface microbial activity. However, at some time during storage the bacterial activity will be sufficient to produce the undesired pigment change to metmyoglobin. Consequently, the change will occur more rapidly with meat of higher initial bacterial activity and/or improper storage conditions.

In present commercial methods where carbon dioxide atmospheres have been used for meat storage, the gas has

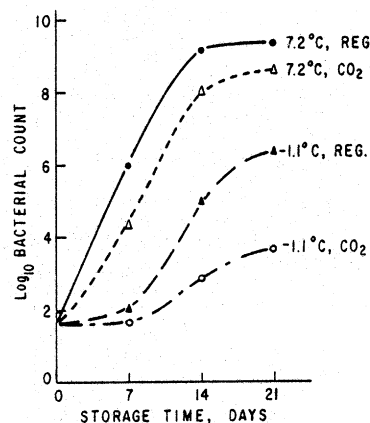


Figure 3. Bacterial growth (plate count) of samples in Figure 2.

been added to the meat container as a solid in pellet or snow form or in the gaseous state. Levels of carbon dioxide in the atmosphere above 5% may be hazardous for human breathing, producing effects on respiration and the nervous system. The inclusion of the dry chemicals within individual packages avoids this hazard to personnel in the immediate area and allows a greater control over long-term gas concentration within the package.

To determine whether CO₂ generating mixtures might be employed to compensate for variations in storage or display temperatures, a subsequent experiment measured the effect of carbon dioxide atmospheres under optimum and nonoptimum temperatures on the color stability of meat. Subprimal cuts of beef (longissimus dorsi muscle), removed several hours following slaughter, were used to prepare the samples. The interaction of temperature and handling and ambient temperature on the various samples were examined. Two storage temperatures, -1.1 and +7.2°C, were used, the lower temperature representing the optimum temperature for meat storage, and the higher representing the upper limit of cooler temperatures, and similar to that temperature found in some household refrigerators. Carbon dioxide atmospheres were generated from ascorbic acid-sodium bicarbonate packets within the gas-impermeable bags containing the meat samples. Control atmosphere samples were also kept within polyethylene bags containing no gas generating system. The effect of both atmospheres on meat color was measured at each temperature. In addition, the effect of handling or nonhandling on each group was examined. Half of the wrapped samples were removed daily from the bag, squeezed several times manually, and returned to the bag along with fresh chemicals if needed. The color values of the unhandled samples are shown in Figure 2. Values for the handled samples were similar.

Under the nonoptimum conditions of storage at 7.2°C and regular atmosphere, the meat surface became brown within the first week. The application of the carbon dioxide atmosphere postponed this color change about 2 days. With the optimum storage temperature of -1.1°C, the meat changed surface color much more slowly with the result that the color values never decreased to those found at 7.2°C. The bacterial counts of the samples are shown in Figure 3, and indicate the importance of temperature in reducing the rate of bacterial growth. The application of the carbon dioxide atmosphere can assist in correcting an adverse rise in temperature. Handling was only a minor factor. The bacterial counts of the handled samples with but one exception were within one log unit of the unhandled samples. To demonstrate the effectiveness of this method under optimum conditions, another sample of meat from this carcass which was wrapped separately and stored under the carbon dioxide atmosphere at -1.1°C was re-

moved after 50 days storage and was found to be completely odor-free. This result may be from an interactive effect of carbon dioxide level and low temperature in controlling microbial growth rates. The meat interior was of excellent color with no metmyoglobin ring formation, and the surface bloomed readily upon exposure to air to give a color value identical with that of the third day following slaughter. The surfaces of this meat sample were trimmed and were evaluated for bacterial growth. The total surface count (log per gram) was approximately 4.0. Colonies in decreasing order of occurrence were described as: catalase-negative Gram-positive cocci; catalase-positive and Gram-negative bacilli; and catalase-positive Gram-positive bacilli.

Although these gas-generating packets might have immediate application in carcass storage where primal cuts are wrapped in gas-impermeable bags or vacuum packed, such packets may also be used with market cuts which are wrapped in gas permeable films. Polyvinyl chloride films used for market wraps have permeability values for carbon dioxide at room temperature from 100 to 3000 cm³ of gas per 100 in.² area per atmosphere per day for a standard 1-mil thickness. Although a larger permeability value for oxygen than for carbon dioxide would be predicted by Graham's law of diffusion, in actuality, the values for oxygen permeability are lower and range from 30 to 2000 cm³. This contrasts with a permeability of approximately 6 to 55 cm³ for carbon dioxide and 1 to 10 cm³ for oxygen transmission for the "nonpermeable" vacuum packaging bags (Johnson, 1974). In our experiments, the plastic film had permeability values of 1200 cm³ for oxygen and approximately 1800 for carbon dioxide, possibly reflecting the differential solubility of the gases in the film's plasticizer.

For a standard steak display package (18 × 24 × 2 cm) the meat is exposed on only one surface to the PVC film for the purpose of permitting the meat to "bloom" or become red. For an effective concentration of 10% carbon dioxide within the package, the packet must release carbon dioxide at a rate which will compensate for the loss of gas through the film. If one employs values of 10% carbon dioxide, permeability of 1800 cm³, etc. and a film thickness of $\frac{3}{4}$ mil, the loss of carbon dioxide through the film would be approximately 0.44 cm³ of carbon dioxide per cm² per day. For this size steak display package, a loss of about 190 cm³/day of carbon dioxide would occur.

Within the package, carbon dioxide is also being produced by the metabolism of the meat tissue but does not remain within the package because of the permeability of the film. Some carbon dioxide will diffuse into the meat liquid, as a function of the partial pressure and temperature. At 10% concentration of the carbon dioxide, the solubility at 0°C is 13 cm³ of carbon dioxide per 100 cm³ of saline solution. One pound of lean meat (70% moisture) would require about 42 cm³ for saturation. Additional carbon dioxide is required for the package void volume. The total volume of the package may be applied advantageously for the calculations of meat plus void volume with little error. For the standard steak display package, a 10% carbon dioxide concentration would require 86 cm³ for the internal volume and 190 cm³ for the film permeability. Under standard conditions, each millimole of bicarbonate can produce a maximum of 22.4 cm³ of carbon dioxide, or 1 g of sodium bicarbonate will produce 267 cm³ of gas with the addition of excess acid. Formulas for predicting the gas exchange through polymeric films in produce have been calculated by Hayakawa et al. (1975).

For the most effective preservation of meat, the carbon dioxide atmosphere should be maintained at the effective concentration throughout the storage period. For the gradual release of carbon dioxide in the gas permeable package, there are several methods of control. Manometric studies have indicated that the time of release of the gas varies with the amount of powder present as does the amount of

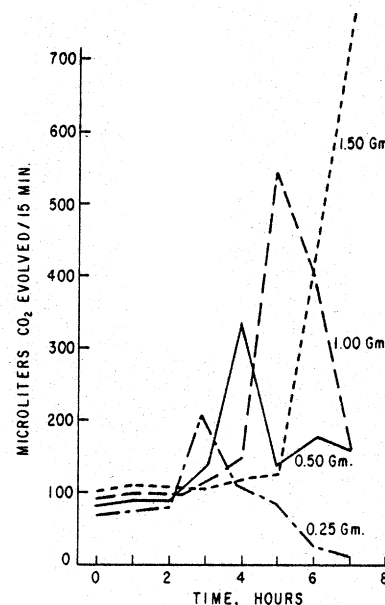


Figure 4. Rate of evolution of carbon dioxide (microliters/15 min) from selected weights of a citric acid-sodium bicarbonate mixture in the presence of saturated water vapor, determined manometrically.

gas released (Figure 4). In our experiments with model systems, there was an initial slow release during a lag period of from 2 to 6 hr followed by a rapid increase in the evolution of carbon dioxide which was produced by the buildup of moisture to the necessary level for reaction. Inasmuch as water is also a product in the reaction, the reaction can be self-perpetuating until completion. The rate of the reaction and evolution of gas can therefore be controlled by a variety of procedures: compartmentalization of the powder packets, variation in hydrophilicity of the acids used, variation in the hydrophobicity of the external packet material, or combinations thereof. Lipophilic acids used as antimicrobial food additives, e.g., sorbic or benzoic acids (Freese et al., 1973), might be considered for solid acids for a delayed reaction time. The amount of water required for initial reaction to take place is a function of the humidity within the package which is a function of temperature. As the temperature within the package rises, the humidity increases, increasing the rate of gas evolution and acting to decrease the concomitant increase in bacterial growth.

Although there is no contact between the meat and the packet, the safety of the chemicals employed could allow variations within legal requirements in the method of application. In this aspect, citric, ascorbic, and carbonic acids are all natural constituents of meat. In a related experiment, meat display trays of experimental design which contained a molded meat drip reservoir and sloped, channeled meat supports were used. Filter paper strips (Whatman no. 1) cut to fit the reservoir were saturated with either citric acid or sodium bicarbonate solutions and allowed to dry. Two strips of paper impregnated with citric acid (0.7 g each) were interleaved with 12 strips of paper impregnated with sodium bicarbonate (0.14 g each). The entire collection contained about 22 mequiv of citric acid and 200 mequiv of bicarbonate and could generate a maximum of about 450 cm³ of carbon dioxide with complete reaction. Surface bacterial counts remained at log value 3 after 8 days storage and then increased to log value 7 after 12 days storage in the experimental trays whereas the bacterial count in the untreated samples was log value 8 after 8 days storage. Percent drip and TBA values for lipid oxidation were similar for the two samples. Similar methods might be employed in the incorporation of the powder mixture in drip absorbent pads.

The decrease in the growth of the total surface microbial

population with increased carbon dioxide levels has been shown by previous investigators (Baran et al., 1970; Clark and Lentz, 1969) to be produced mainly by the reduction of growth of pseudomonads on the meat surface. Pseudomonads, although aerobic, can grow effectively at oxygen concentrations of 0.8%, but are inhibited by increasing concentrations of carbon dioxide, with 10% carbon dioxide causing 44% inhibition in the growth of *Pseudomonas* 1482 (Ledward et al., 1971). As demonstrated by these authors, it is easier under industrial conditions to increase the carbon dioxide concentration to 10% than to decrease the oxygen concentration to less than 0.4%. The inhibition by carbon dioxide is also more effective at a given concentration as temperature is decreased.

King and Nagel (1967) investigated various mechanisms and factors regulating the growth of pseudomonads that could be influenced by carbon dioxide levels. The inhibition did not appear to be produced by alterations in oxygen tension, pH, or ionic strength of the substrate solutions. It appeared that specific enzymes involved in the catabolism of the various substrates examined were influenced to different degrees by carbon dioxide. More recently (King and Nagel, 1975), these authors concluded that the action of carbon dioxide on *Pseudomonas aeruginosa* was to limit the rate of growth by a mass action inhibition on certain decarboxylating enzymes, particularly isocitric and malate dehydrogenases. Incubation temperature has also been shown to alter the proportional utilization of the Entner-Doudoroff and hexose monophosphate pathways of glucose catabolism in *Pseudomonas fluorescens* through regulation of the growth limiting concentration of glucose (Palumbo and Witter, 1969). Although the substrates in meat that are actually utilized by the pseudomonads are unknown and may vary between meat samples, similar types of regulation of growth by carbon dioxide concentration may have occurred in these experiments.

As a result of inhibited pseudomonads growth, the slower growing lactobacilli and microbacterium flora which can

grow in low oxygen and high carbon dioxide are predominant (Ledward et al., 1971). These species appear to have little effect on the formation of metmyoglobin during storage and are not a major concern in fresh meat spoilage.

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